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Influence of vitamin C supplementation on lead-induced histopathological alterations in male rats

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ABSTRACT

This study is intended to evaluate the efficacy of vitamin C (VC) in ameliorating the detrimental effects of long-term lead intoxication on the liver, kidneys, brain and testes as assessed by histopathology. A total of forty male Wistar rats (six-weeks-old) was divided into 4 groups: control group; lead-acetate (PbAc)-treated group (20 mg PbAc/kg bwt); PbAc+VC-treated group (20 mg PbAc/kg bwt plus 20 mg VC/kg bwt); and VC-treated group (20 mg VC/kg bwt). The Experimental period was lasted for 60 successive days in which PbAc was administered once daily while VC was supplemented every other day using intra-gastric intubation. At the end of the experimental period, all rats were sacrificed and pathological examinations were performed. Control and VC-supplemented rats showed normal liver, kidney, brain, and testes histology. In contrast, the liver of PbAc-intoxicated rats exhibited degenerated hepatocytes and portal inflammatory cell infiltrations. The kidneys showed degenerated glomeruli and formation of karyomegalic cells containing intranuclear inclusions in the proximal tubular epithelium. Cerebellar edema, cerebral satellitosis and encephalomalacia observed in the brain. Testicular tissues showed arrest of spermatogenesis and interstitial edema. Co-administration of VC with PbAc diminished the severity of pathological changes and reduced the number of affected organs compared to PbAc-intoxicated rats. These results show that low level of VC ameliorated and mitigated the adverse pathological impacts of chronic lead toxicity.

1. Introduction

Lead (Pb) toxicity is probably the most common form of heavy metal intoxication. It is well-documented as one of the most dangerous and insidious poisons. Its continuous environmental and occupational exposure may contribute to renal, nervous, hepatic, hematological and reproductive disorders in man and animals (Flora et al., 2006; El-Sayed and El-Neweshy, 2009, Ashry et al., 2010). The absorbed Pb is conjugated in the liver and passed to the kidney, where a small quantity is excreted in urine and the rest accumulates in various body organs and affects many biological activities at the molecular, cellular and intercellular levels, which may result in morphological alterations that can remain even after Pb levels have fallen (Jarrar, 2003; Sidhu and Nehru, 2004; Taib et al., 2004; Flora et al., 2006). Lead-induced oxidative stress or disruption of prooxidant/antioxidant balance in blood and other soft tissues has been postulated to be the major mechanism of Pb-associated tissue injury (Pande et al., 2001; Flora et al., 2003). It causes oxidative stress by inducing the generation of reactive oxygen species (ROS) (Gurer and Ercal, 2000), increasing the level of lipid peroxidation and thiobarbituric acid-reactive substances (Adonaylo and Oteiza, 1999; Upasani et al., 2001; Flora et al., 2003; Ashry et al., 2010), and inhibiting the activity of many antioxidant enzymes, including glutathione (Sidhu and Nehru, 2004; Flora et al., 2006; Ashry et al., 2010). Consequently, Pb alters the antioxidant defense system of cells resulting in pathophysiological events in various body organs. Hence, the therapeutic strategy of strengthening the cell's antioxidant capacity may fortify the long-term effective treatment of Pb poisoning. This may be achieved through exogenous supplementation of antioxidant molecules as an alternative to chelation therapy (Gurer and Ercal, 2000; Flora et al., 2003; Ashry et al., 2010).

Vitamin C (Ascorbic acid, VC), a known chelating agent with non-enzymatic antioxidant features, was widely reported to have the ability to protect cells from oxidative stress (Patra and Swarup, 2004; Palaniappan et al., 2005; Rai et al., 2009). It is the most important free radical scavenger in extracellular fluids, trapping radicals in the aqueous phase and protecting biomembranes from peroxidative damage (Patra and Swarup, 2004; Rai et al., 2009). In Pb-exposed rats, VC has the ability to chelate Pb, with similar potency to that of EDTA. It may increase urinary elimination of Pb

Abbreviations: AAS, atomic absorption spectrophotometry; HE, hematoxylin and eosin; HPF, high power field; NaAc, sodium-acetate; NIH, National Institutes of Health; Pb, lead; PbAc, lead-acetate; ROS, reactive oxygen species; VC, vitamin C; Δ-ALAD, δ-amino levulinc acid dehydratase

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and reduce its hepatic and renal burden (Dawson et al., 1999; Simon and Hudes, 1999). Furthermore, VC significantly reduced the lipid peroxidation levels of liver and brain, while increased the catalase activity (Patra et al., 2001; Rai et al., 2009). Its supplementation in Pb-intoxicated rats was also associated with serum biochemical alterations in the hematological system and drug metabolizing enzymes. It significantly reduced blood, liver and kidneys Pb, blood zinc protoporphyrin and free radical levels while increasing the activity of blood δ-amino levulinc acid dehydratase (Δ-ALAD) (Flora and Tandon, 1986; Vij et al., 1998). There is a lack of experimental research papers concerning the protective effect of VC against long-term Pb toxicity at the histopathological levels. Thus, this study aimed to investigate the ameliorative effect of VC against chronic Pb-associated direct tissue damage in the liver, kidneys, brain and testes of male rats by observing histopathological alterations.

2. Materials and methods

2.1. Chemicals

Lead-acetate [$(C_2H_3O_2)_2Pb \cdot 3H_2O$] (PbAc) and sodium-acetate [$CH_3COONa \cdot 3H_2O$] (NaAc) were obtained from Sigma Chemical Co. (St. Louis, MO). Vitamin C (ascorbic acid) was obtained from Pharco Pharmaceutical Company, Alexandria, Egypt. All other chemicals and reagents used were of analytical reagent grade.

2.2. Heavy metal and vitamin preparation

A 0.5% Pb solution was prepared by dissolving 5 g of PbAc in 1000 mL of distilled acidified water to prevent the formation of Pb precipitate. A corresponding solution of NaAc containing equivalent amount of acetate was prepared and used as a vehicle for the control. Vitamin C dissolved in saline and used freshly in experimental animals.

2.3. Animals and experimental design

Forty male Wistar rats (six-weeks-old and weighing 100 ± 20 g) were obtained from the Laboratory Animal Breeding Colony, Faculty of Agriculture, Alexandria University. The animals were housed in plastic cages free from any source of chemical contamination under controlled conditions with an ambient temperature range of 22 ± 2 °C, relative humidity of 50 ± 5% and a 12 h light-cycle with free access to commercial food and water. The standard laboratory diet is composed of 161 g/kg protein, 36.4 g/kg fat, 41.1 g/kg fiber and 12.1 MJ metabolizable energy, and purchased from Damanhur Feed Co. (Behera, Egypt). Soft wood shavings were used for bedding and changed during the cleaning of cages on alternate days. All animals received humane care in compliance with the Animal Care guidelines of the National Institutes of Health (NIH), and the local committee approved the design of the experiments.

After an acclimatization period of 10 days, the pubertal rats were randomly assigned into 4 groups (ten rats per group):

Group A (Control rats) was given 15 mg NaAc/kg bwt, intra-gastrically once daily;
Group B (PbAc-treated rats) received 20 mg PbAc/kg bwt, intra-gastrically once daily (Institóris et al., 1999);
Group C (PbAc+VC-treated rats) received 20 mg PbAc/kg bwt, once daily and were concurrently supplemented with 20 mg VC/kg bwt, every other day 30 min before PbAc administration using intra-gastric intubation;
Group D (VC-treated rats) received 15 mg NaAc/kg bwt, intra-gastrically once daily and were concurrently supplemented with 20 mg VC/kg bwt, every other day 30 min before NaAc administration using intra-gastric intubation.

The timing and dose of vitamin C pretreatment have been selected on the basis of previous reports, and to build antioxidant pool in animal body before heavy metal exposure (Rai et al., 2009; Mor and Ozmen, 2010). The experiment was conducted for 60

Table 1
Incidence and severity of histopathological lesions in the liver, kidneys, brain and testes of lead-acetate (PbAc)-treated group (20 mg/kg bwt/24 h, orally) (group B), and PbAc+vitamin C (VC)-treated group (20 mg PbAc/kg bwt/24 h, orally plus 20 mg VC/kg bwt/48 h, orally) (group C).

Organ/Lesion	Incidence and Severity of Histopathological Lesions							
	Group B (PbAc-treated rats)				Group C (PbAc+VC-treated rats)			
	Normal (−)	Mild (+)	Moderate (++)	Severe (+++)	Normal (−)	Mild (+)	Moderate (++)	Severe (+++)
Liver								
Portal inflammatory cells infiltration	0	1	7	2	4	5	1	0
Degenerated hepatic cells	0	3	6	1	5	3	2	0
Kidneys								
Damaged glomeruli	0	4	3	3	4	5	1	0
Degenerated cortical tubules	0	2	3	5	1	6	1	2
Necrotic cortical tubules	3	2	2	3	9	1	0	0
Brain								
Cerebellar white matter edema	2	2	3	3	9	1	0	0
Cerebrocortical necrosis(encephalomalacia)	1	2	5	2	10	0	0	0
Cerebrocortical satellitosis	0	2	7	1	4	2	4	0
Cerebrocortical neurophagia	1	3	5	1	10	0	0	0
Cerebrocortical edema	0	1	7	2	5	4	1	0
Perivascular monocytic aggregations in cerebral cortex	0	3	7	0	7	2	1	0
Testes								
Aspermia	0	0	1	9	9	1	0	0
Shrunken and wavy outlined seminiferous tubules	0	0	2	8	8	2	0	0
Binucleated giant cells formation	7	3	0	0	10	0	0	0
Intertubular edema	0	0	0	10	2	2	6	0
Damaged germinal cells	0	0	1	9	4	4	2	0

*Number of rats with lesions per total examined (10 rats per group).